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A Multicenter Study of Patients with Timothy Syndrome

Mark A. Walsh, MD [1,2], Christian Turner, MD [3,4], Katherine W. Timothy, BS [5], Neil Seller, MD [3], Dominic L. Hares, MD [6], Andrew F. James, DPhil [7], Jules C. Hancox, FBPhS, FRSB [7], Orhan Uzun, MD [8], Dean Boyce, MD [9], Alan. G. Stuart, MD [1,2], Paul Brennan, MD [10], Caroline Sarton, BS [11], Karen McGuire, PhD [11], Ruth A. Newbury-Ecob, MD [12], Karen Mcleod, MD [13]

[1] Bristol Royal Hospital for Children, [2] Bristol Heart Institute, University Hospital Bristol, Bristol, United Kingdom

[3] Department of Congenital Cardiology, Freeman Hospital, Newcastle upon Tyne, United Kingdom

[4] Children's Hospital at Westmead, Sydney, Australia

[5] Katherine W Timothy, Clinical Coordinator (retired), Children's Hospital Boston Harvard Medical School, Boston, Massachusetts

[6] Department of Cardiology, The Yorkshire Heart Centre, Leeds General Infirmary, Leeds, United Kingdom

[7] School of Physiology, Pharmacology and Neuroscience Cardiovascular Research Laboratories, University of Bristol

[8] Department of Cardiology, University Hospital Wales, Cardiff

[9] Department of Plastic Surgery, University Hospital Wales, Cardiff

[10]Department of Clinical Genetics, Freeman Hospital, Newcastle upon Tyne, United Kingdom

[11] Oxford Medical Genetics Laboratories, Cardiac Service, Oxford University Hospitals NHS Trust, The Churchill Hospital, Oxford

[12] Department of Clinical Genetics, University Hospital Bristol, Bristol, United Kingdom

[13] *Department of Cardiology, Royal Hospital for Sick Children, Glasgow, United Kingdom*

Correspondence: Mark Walsh, Bristol Royal Hospital for Children, Upper Maudlin Street, Bristol, BS2 8BJ

Email: mark.walsh@UHBristol.nhs.uk

Phone: 0117 3428852

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Timothy Syndrome: TS

Automated External Defibrillator: AED

Implantable Cardiac Defibrillator: ICD

Abstract

Background - Timothy syndrome (TS) is an extremely rare multisystem disorder characterized by marked QT prolongation, syndactyly, seizures, behavioral abnormalities, immunodeficiency and hypoglycemia. There is a propensity to develop malignant arrhythmias at a young age, with many patients requiring defibrillator implantation.

Methods – This multicenter study looks study looks at all patients diagnosed with TS in the United Kingdom over a 24-year period. Fifteen centers in the British Congenital Arrhythmia Group network were contacted to partake in the study.

Results - Six patients with Timothy syndrome were identified over a 24-year period (4 boys and 2 girls). Five out of six were confirmed to have a *CACNA1C* mutation (p.Gly406Arg) and the other patient was diagnosed clinically. Early presentation with heart block, due to QT prolongation was frequently seen. Four are still alive, two of these have a pacemaker and two have undergone defibrillator implantation. Five out of six patients have had a documented cardiac arrest with three occurring under general anesthesia. Two patients suffered a cardiac arrest while in hospital and resuscitation was unsuccessful, despite immediate access to a defibrillator. Surviving patients seem to have mild developmental delay and learning difficulties.

Conclusion – Timothy syndrome is a rare disorder with a high attrition rate if undiagnosed. Perioperative cardiac arrests are common and not always amenable to resuscitation. Longer-term survival is possible, however patients invariably require pacemaker or defibrillator implantation.

Introduction

Timothy syndrome (TS) is an extremely rare multisystem disorder caused by a *de novo* mutation in the alternatively spliced exon 8A affecting *CACNA1C,* the gene encoding the $Ca_V1.2$ calcium channel (p.Gly406Arg).[1-3] The $Ca_V1.2$ channel is critical for the plateau phase of the cardiac action potential, cellular excitability, excitation–contraction coupling, and regulation of gene expression.[4, 5] In TS the mutation causes impaired inactivation of open-state voltage-dependent L-type calcium channels, which results in a relatively sustained inward Ca^{2+} current.[6] It is characterized by marked QT prolongation, syndactyly, immune deficiency, seizures, congenital heart defects, cognitive abnormalities, learning difficulties and intermittent hypoglycemia.[2, 7] Timothy syndrome type 2 (TS2) is used to describe patients with the above phenotype but without syndactyly. Mutations in exon 8, rather than exon8A (p.Gly402Ser and p.Gly406Arg) have been described in TS2; differential expression of exon 8 / 8A in the heart and brain are thought to account for the different phenotypes.[3] It is a remarkably genetically homogenous disorder, with most reported phenotypes accounted for by these two genotypes.[3]

From the limited case reports available, both TS1 and TS2 usually present at a young age. The two most common modes of presentation are heart block as a result of QT prolongation and cardiac arrest.[8] General anesthesia appears to be a particularly vulnerable time for these patients, with some undiagnosed patients presenting with a cardiac arrest during syndactyly surgery.[9] Without treatment survival is unlikely due to the propensity for malignant arrhythmias. With improved

understanding how antiarrhythmic medications affect the heart in TS, and better pacemaker / defibrillator therapies, survival into young adulthood is also possible.[3] In this study we looked at all patients who were diagnosed with TS1 and TS2 in the United Kingdom over a 24-year period, which represents the only multi-center published to date.

Methods

This is a multicenter study conducted on behalf of the British Congenital Arrhythmia group (BCAG), which is a network of all hospitals managing paediatric arrhythmias and inherited cardiac conditions in the United Kingdom. The network consists of 15 centers, 12 of which perform paediatric cardiac surgery / interventions, and 3 of which are non-surgical. All centers have a paediatric cardiologist with a specialist interest in inherited cardiac conditions. The study was coordinated through Bristol Royal Hospital for Children. Research and ethics approval was obtained prior to commencing the study. Representatives for all centers were contacted to contribute cases. Any patients who were either born in, or currently reside in the United Kingdom were eligible for inclusion in the study.

Patient Demographics

Basic demographic details were collected from a combination of cases notes review, discussion with physicians, and discussions with TS patients' families. In particular we were interested in the antenatal history, age at presentation, the presence of heart block, QTc at presentation, and medications. We collected data on interventions such as placement of pacemakers, ICDs, the timing of these interventions, and occurrence of appropriate / inappropriate shocks. Also, we looked at any non-arrhythmia related episodes of cardiovascular compromise.

Genetics

Most genetic testing inherited channelopathies are currently performed at Oxford Genetics Laboratories in the United Kingdom. We reviewed the database at this laboratory and there were no additional cases found other than the ones provided by the BCAG members. Other genetics laboratories were contacted to get specific details on mutations and to obtain illustrations. Given the rarity of this disorder and the high attrition rate, we thought that patients should be included if there was a strong index of suspicion, in the absence of genetic confirmation of the condition. Information was also requested on immediate family members, although genetic testing was not performed on any of them.

Exon 8 of the *CACNA1C* gene (accession sequence NM_001167625.1) was amplified by PCR from genomic DNA (as supplied) using a readymade mastermix, KAPA2G Fast HS Readymix, supplied by Kappa Biosystems. Bidirectional fluorescent dideoxy sequencing was performed using Applied Biosystems Big Dye Terminator v3.1 kit followed by capillary electrophoresis on the Applied Biosystems 3730. Analysis involved manual interrogation at nucleotide position c.1216. Variant description is according to Human Genome Variation Society (HGVS) nomenclature. Internal quality control samples were run for each test, including negative (water blank), positive (DNA from the proband) and normal (previously assigned normal sequencing control). The test was undertaken using two non-overlapping primer sets so as to eliminate the possibility of allele drop out due to their being a polymorphism in the primer binding site.

1

Results

All 15 centers in the BCAG network were contacted and the response rate was 100%. A total of six patients with TS were identified at five centers from January 1992 to April 2016; ten centers confirmed that they didn't have any cases. During this time period in the United Kingdom there were approximately 16 million live births, giving an approximate incidence of 1.5 in 10^8 births per year.[10] In total there were 4 boys and 2 girls. Two of the patients were monozygous twins (both male). At the time of writing 4 out of 6 patients are still alive (table 1). Of the patients that are alive, two have pacemakers and two have had a defibrillator placed (table 2). A summary of the clinical course of all six patients is outlined below. There was no significant family history of sudden death in any of the patients. We were able to ascertain that 4 out of 5 parents of index cases were assessed clinically and did not demonstrate any clinical phenotypical features of TS. None of the parents of index cases underwent genetic testing.

Patient # 1

This patient was delivered at 28 weeks by emergency caesarian section (weight 980 grams) for fetal bradycardia. The baby initially required ventilation for respiratory distress syndrome and remained intubated for four weeks after delivery. There was marked prolongation of the QT interval (QTc: 600 msec), which was causing 2:1 heart block with hemodynamic instability. An attempt was made to slow the heart rate with intravenous propranolol, however propranolol administration caused further prolongation of the QT interval (Figure 1). A low dose of isoproterenol (0.01 mcg/kg/min) was commenced which resulted in some improvement. Mexiletine (4 mg/kg, Q8 hourly) was added at four weeks of age which resulted in further improvement, however intermittent 2:1 block was still present (Figure 2). Genetic testing confirmed the presence of a *CACNA1C* mutation. At eight weeks of age during induction of anesthesia for a Hickman line, the patient had an episode of ventricular fibrillation that required cardioversion. The isoproterenol infusion was continued at rates of 50 – 250 ng/kg/min until an epicardial pacemaker could be undertaken; this was performed at 12 weeks of age at a weight of 2.2 kg. Propranolol 1 mg / kg was also commenced, in addition to Mexiletine, once satisfactory pacing was achieved.

The patient was discharged after a hospital stay of three months. At 12 months of age she was admitted to hospital with gastroenteritis and suffered another cardiac arrest. Cardiopulmonary resuscitation was performed immediately and an automated external defibrillator (AED) was attached; the rhythm was judged not to

require cardioversion. The patient recovered after five minutes of resuscitation. There was mild hyperkalemia at the time of the cardiac arrest (6.7 mmol/L) and blood glucose was confirmed to be normal. At 15 months of age one of the epicardial leads became fractured. A transvenous pacemaker was placed without any complications. The patient's current medications consist of mexiletine and propranolol. Regular Holter monitors have not demonstrated any arrhythmias. She is currently managed with a pacemaker and an AED, however consideration will be given to upgrade to an ICD when she is of sufficient size.

Patient #2

Patient #2 was born at 30 weeks gestation (weight 1.5 kg) due to premature labor. The patient was noted to be in heart block on fetal echocardiograms. There was prolongation of the QT interval at birth (600 msec) with 2:1 heart block. The patient was noted to have syndactyly of the index, middle and ring fingers on both hands. Genetic testing confirmed the presence of a *CACNA1C* mutation. There was a patent ductus arteriosus and a ventricular septal defect which both closed spontaneously. The patient was maintained on atenolol initially and subsequently nadolol. A pacemaker was placed at two years of age. An episode of ventricular fibrillation occurred during induction of anesthesia that was treated with DC cardioversion. The patient was noted to have seizure activity on transfer to the ward afterwards, however made a good recovery and was discharged. During a respite hospice admission, he had another cardiac arrest that was cardioverted successfully by an AED. He was subsequently transferred to hospital and made a good recovery, although he did have some ongoing seizures. Unexpectedly, he had a sudden unprovoked cardiac arrest while still in hospital. Immediate cardiopulmonary resuscitation was commenced. His initial rhythm was pulseless electrical activity, which was followed soon afterwards by asystole. His pacemaker was functioning appropriately at the time and pacing at its lower limit. Blood glucose was checked around the time of cardiac arrest, which was normal.

Patient #3

This patient is likely one of the oldest patients alive with TS (24 years old). She was born at term with a weight of 3 kg. She had an out of hospital cardiac arrest at one year of age. She was successfully cardioverted from ventricular fibrillation and transferred to hospital. She had a QT interval of 600 msec, however it was not clear whether this was related to the cardiac arrest. There was no evidence of atrioventricular block. Other than a patent ductus arteriosus she had a structurally normal heart. She never had any seizures. She was treated with atenolol 1 mg / kg twice daily. Four years later she had another out-of-hospital cardiac arrest that resulted in a neurological injury. An epicardial defibrillator was placed at 5 years of age using pericardial patches and an endocardial pace-sense lead. She had an appropriate shock from this device at 8 years of age. Genetic testing confirmed the presence of a *CACNA1C* mutation. She had had multiple device changes and lead extractions. Currently she is living independently; she has mild learning difficulties and mild residual weakness as a result of her previous neurological injury.

Patient #4

A 13-month-old boy was admitted for bilateral syndactyly release. He had soft systolic heart murmur, however it is not clear whether an echocardiogram was performed. There was syndactyly of the little and ring fingers. Anesthesia was induced and maintained with sevoflurane. One hour into the procedure, it was noticed that the patient was in ventricular bigeminy and T-wave alternans; all other parameters remained unchanged so the operation continued. One hour later, the patient had a cardiac arrest due to ventricular fibrillation, which was successfully cardioverted. Two further cardioversions were performed due to a reoccurrence of ventricular fibrillation. A narrow complex tachycardia followed, with good cardiac output. Subsequently a bradycardia ensued with no palpable pulse. Further attempts at resuscitation were unsuccessful and 75 minutes after the initial arrest, resuscitation was discontinued. The post mortem demonstrated a patent ductus arteriosus, a myocardial bridge over the right coronary artery, myocardial fibrosis and endocardial fibroelastosis. Subsequent examination of the ECG strip confirmed the presence of a prolonged QT interval, which was unknown to the treating clinicians. Genetic testing was not performed on this patient

Patient #5

Twin I of monozygous twins, long QT syndrome was first suspected when transient 2:1 atrioventricular block was noticed as a neonate. Serial ECGs showed persistent QTc prolongation of up to 554 ms and, despite the absence of symptoms, he was started on treatment with nadolol 1 mg/kg/day at the age of two years. An episode of syncope at five years of age prompted implantation of a loop recorder. On induction of anesthesia for this procedure he developed torsade de pointes, followed by sinus rhythm with T wave alternans (figure 5). A further episode of torsade de pointes was then documented, and an ICD was subsequently implanted for recurrent syncope despite adequate beta blockade. The ICD has delivered appropriate therapy on several occasions although there has been only one recurrence of arrhythmia (precipitated by a fire alarm at school) since the nadolol administration was changed to 0.5 mg/kg twice daily. Twin I's QTc intervals have ranged from 522 to 554 ms. His other problems have included an inguinal hernia, congenital dysplasia of the hip, strabismus requiring glasses and otitis media with effusions, managed with grommets. His joints are hypermobile, and he wears splints to support his ankles and feet. He has global developmental delay, with delay in gross motor, fine motor skills, with a limited vocabulary, communicating primarily by signing. At a recent assessment, he was on the 3rd centile for height (just below the lower limit of his parental range), and on the 25th centile for weight. At birth he was between the 50th and 75th centile for weight. Twin I does not have congenital heart disease. He does not have syndactyly. He has bilateral epicanthic folds but otherwise no facial dysmorphism.

Subsequent molecular analysis demonstrated the pathogenic *CACNA1C* gene mutation c.1216G>A (pGly406Arg) in mosaic form in both twins. This mutation was absent, however, from both parents, suggesting that the mutation arose as a *de novo* event. Testing of DNA extracted from buccal smear samples confirmed that the mutation was present in both twins in mosaic form (Figure 4).

Patient #6

Twin II presented with cyanosis and was diagnosed with pulmonary atresia and ventricular septal defect. This was initially palliated with arterial shunts before repair with a Rastelli procedure at four years of age. His ECGs also showed a long QTc interval (range 493 to 555 ms), and he has been treated with nadolol since the age of two years. Other than transient 2:1 AV block he has not had overt arrhythmia. Other medical problems consisted of bilateral inguinal hernias and undescended testes requiring repair. Following one of the cardiac surgical procedures, he developed a Klebsiella urinary tract infection. He subsequently also developed Morexella endocarditis. Failure-to-thrive has recently been treated with growth hormone. At two years of age, he was found to be intolerant to lactose and soya. Following exclusion of these, his slow growth improved. Whilst twin II does not have strabismus, he has previously had abnormal eye movements. Investigation with electroencephalogram was normal. He weighed 1599 g at birth (10^{th} centile). At a recent assessment, he was below the 3^{rd} centile for height (3.85 STD below the mean) and weight just below the 3^{rd} centile. He has hypermobile joints. Like his brother, twin II also has global development delay, particularly in the areas of speech and language, but also in fine and gross motor skills. He does not have syndactyly; he also has bilateral epicanthic folds but otherwise no facial dysmorphism. He has postural plagiocephaly. He currently has a single chamber pacemaker, however has not required placement of an ICD.

Discussion

This is the first multicenter study looking at outcomes for patients with TS. All patients in the series had the same mutation (p.Gly406Arg) affecting the *CACNA1C* gene. Early presentation is common and pacing / defibrillator placement is usually required. Patients are at high risk of ventricular arrhythmias, particularly during general anesthesia, indeed this was the initial presentation in one case. It is clearly a complex multisystem disorder, some of the facets of which are poorly understood: this is evident by the fact that 2 patients died in hospital despite good apparent resuscitation and appropriate cardioversion. Other patients have had successful cardioversion following cardiac arrest, demonstrating that defibrillator therapy can be life saving. Neurological impairments are frequent, most commonly seizures, as are mild learning difficulties. This study also demonstrates that with appropriate medical and device therapy, survival into adulthood is possible.

As with other reports, this study demonstrates the genetic homogeneity of TS with all phenotypes resulting from the same mutation (p.Gly406Arg), exon 8A for TS1 and exon 8 for TS2. It is a missense mutation in the pore-forming region of the $Ca_V1.2$ channel that is highly conserved across various species.[3, 11] The other mutation described only in TS2 is a missense mutation, (p.Gly402Ser / exon 8) and presents predominantly with cardiac involvement; we did not see this mutation in our study. The $Ca_V1.2$, transmembrane segment 6 of domain I (Figure 6), can be encoded by two mutually exclusive exons, 8 and 8A.[3] The two phenotypes and the presence or absence of syndactyly are thought to be the result of differential

expression of exons 8 / 8A, for instance exon 8 represents 80% of mRNAs in heart and brain. It is possible that some patients with TS2 may be undiagnosed due to the absence of syndactyly, and succumb early in life to malignant arrhythmias.

Three patients in our study with TS1 demonstrated the abovementioned missense mutation (pGly406Arg) in exon 8A. The other two patients with TS2 (pGly406R mutation / exon 8) did not demonstrate as severe a phenotype due to a mosaic mutation. Mosaicism occurs as a result of genetically distinct populations in the somatic and germline tissues with heterogeneous expression, which may not follow Mendelian rules of inheritance.[12, 13] With patient #5 and #6, the mutation was demonstrated to be absent from both parents thus occurring as a *de novo*, *post-zygotic* event, after fusion of oocyte and spermatozoon but before cleavage of the zygote into monozygotic twin embryos. There are only two other published reports of mosaicism in TS, and these have described siblings with a more severe phenotype inherited from a phenotypically normal parent with a mosaic mutation.[3, 12] The presence of mosaic mutations underlies the importance of testing tissues other than peripheral lymphocytes: for instance, buccal swabs in parents of index cases. This has important implications in counseling for future pregnancies.

Mutations affecting the $Ca_V1.2$ channel have wide-reaching consequences. In addition to the electrical abnormalities, the presence of syndactyly, immune deficiency, seizures, congenital heart defects, cognitive abnormalities, learning difficulties and hypoglycemia underlie how different organ systems can be affected.

With respect to the developing brain, L-type calcium channels are essential for linking electrical events to the activation of signaling pathways that regulate the development and function of neurons.[14] Krey et al demonstrated that the impaired inactivation of the L-type calcium causes dendrite retraction when neurons are stimulated electrically. The mechanism of action however is thought to be independent of calcium influx into the cell; rather, it may be due to a conformational change in the last transmembrane spanning region of the first repeat of $Ca_V1.2$, controlling Gem and RhoA signaling cascades.[15] This provides an insight into how mutations in the *CACNA1C* gene might cause autistic traits and some of the other neurological abnormalities in this condition. One of our patients had documented recurrent hypoglycemia, although he was not hypoglycemic at the time of his cardiac arrest (patient #2). The mechanism of hypoglycemia likely relates to the effect of activation of L-type voltage-dependent calcium channels on the B-cells of the pancreas.[16] It is possible that some of the unsuccessful resuscitations in this study may have been related to hypoglycemia. It was not known that the patient who died during syndactyly surgery had a prolonged QT interval until reviewed retrospectively. It is not usual practice for patients undergoing minor procedures to have a preoperative ECG. This underlies the importance for specialists dealing with syndactyly to be aware of this very rare condition, to obtain specialist input, and to have the procedure performed in a specialist centre.

With respect to inactivation of calcium channels, it is thought that loss of voltage dependent inactivation is the predominant effect in TS.[3, 4, 6] Recent studies however

have suggested that Ca^{2+} / Calmodulin dependent inactivation (CDI) also plays an important role: Dick et al have shown how the mutations p.Gly402Ser and p.Gly406Arg have different effects on CDI; also they showed a non-linear effect of TS gene expression on arrhythmia inducibility.[17] This may explain why mosaic patients, such as the two seen in our study, have a comparatively milder phenotype. In terms of therapeutics it also means that we may not need to block all of the mutant TS channels to prevent arrhythmogenesis. There are 2 reported cases where verapamil has been used to treat TS. Jacobs report a reduction in the burden of ventricular tachycardia in a TS2 patient with the mutation Gly402Ser.[7] In another patient with TS1 due to a mosaic p.Gly406Arg mutation, verapamil actually increased the arrhythmia burden.[12] This illustrates how the same medication may produce different effects depending on the mutation and its level of expression. All of the surviving patients in our study mere managed in the longer term with β-blockers. As a newborn, patient #1 demonstrated lengthening of the QT interval with intravenous propranolol, thus perpetuating 2:1 block. This paradoxical QT prolongation with β-blocker administration has been seen in other studies and the cause remains unclear.[18] Isoproterenol seemed to alleviate 2:1 block, however the improvement was intermittent and ultimately pacing was required. It does seem counterintuitive that most patients with TS are managed chronically with β-blockers given their effect on the newborn QT interval.[18] Mexiletine resulted in shortening of the QT interval in patient #1, which has been seen in other studies.[18, 19] Mexiletine works by inhibition of the late inward sodium channel current without having an effect on the inward calcium current.[18] This current plays a role in the

rate adaptation of ventricular repolarization. Inhibition of this current results in a blunted bradycardia dependent QT prolongation.[18]

The presence of 2:1 block as a neonate usually indicates that early pacemaker placement will be required. The benefit of a pacemaker is two-fold; firstly, it improves cardiac output by preventing 2:1 block. Secondly, it prevents heart rate dependent variation of the QT interval.[18] Whether it is performed endocardial or epicardial will depend on the patients weight and institutional practice. The benefit from pacing will usually outweigh any possible morbidity from the procedure once a baby has reached around 2.5 kg. Defibrillator placement at a young age will usually require a much higher burden of proof that there will be definite benefit. There is a high risk of device malfunction and inappropriate shocks at this age using an epicardial approach.[20] It is also possible that defibrillation in some cases may be ineffective; two patients in our study died in hospital despite appropriate external defibrillation. Another patient (patient #1) had a cardiac arrest which was judged not to require cardioversion by an AED; this has also been reported in other patients.[21] Current recommendations are that an ICD may be considered in high-risk patients such as those with TS without symptoms.[22] Hypoglycemia is common in patients with TS and this may account for some cardiac arrests, however this was not definitely deemed to be a causative factor in any cases in our study. Bradycardia and asystole, despite presence of a pacemaker, was seen in two patients in our study. Two patients underwent defibrillator placement at 5 years of age, and both have received at least one appropriate shock. As patients get older the morbidity

from defibrillators decreases, so implantation for primary prevention may be appropriate.

Conclusions

This is a multicenter study looking at all cases of TS patients in the United Kingdom over a 24-year period. If untreated, the mortality is high; however, with appropriate medical and device therapy, longer-term survival is possible. Due to the multiple systems affected by this disorder, not all deaths are related to tachyarrhythmias; hence, careful consideration must be given prior to implanting defibrillators in smaller patients.

Table 1. Baseline demographics

Patient	Age[#]	Gestation	Pregnancy	Delivery	Gender	2:1 HB	QTc * (msec)	Syndactyly	Seizures	Other diagnosis
1	1 day	28/40	Normal	EmLSCS	F	Yes	569	Yes	Yes	PDA
2	1 day	30/40	Heart Block	Normal	M	Yes	600	Yes	Yes	PDA, VSD
3	1 year	Term	Normal	Normal	F	No	NA	Yes	No	PDA
4	13 mths	Term	Normal	Normal	M	No	NA	Yes	No	PDA, EFE, RCA bridge
5	1 week	32/40	Twin	LSCS	M	Yes	554	No	No	Inguinal Hernia
6	1 day	32/40	Twin	LSCS	M	Yes	555	No	No	Pulmonary atresia

[#]Age at presentation, *Rate corrected QT (QTc) interval at time of diagnosis (Bazett's correction), HB: heart block, LSCS: lower segment caesarian section, PDA: patent ductus arteriosus, VSD: ventricular septal defect, EFE: endocardial fibroelastosis, RCA: right coronary artery

Table 2. Pacemaker and Defibrillator device information

Patient	Alive	Current Device	Current Age (years)	Age at first Device	Appropriate ICD shocks	Cardiac Arrest
1	Yes	PPM	1 yr, 7 mths	PPM @ 8 wks	NA	VF (related to anesthetic), Bradycardic arrest
2	No	NA	-	PPM @ 2 years	NA	Asystolic arrest on cardiology ward
3	Yes	ICD	24	ICD @ 5 years	Yes	Multiple appropriate shocks
4	No	NA	-	None	NA	torsade de pointes, not resuscitatable
5	Yes	ICD	7	ICD @ 5 years	Yes	Multiple appropriate shocks
6	Yes	PPM	7	PPM @ 4 years	NA	None

PPM: permanent pacemaker, ICD: Internal Cardiac Defibrillator,

Figure 1: Patient #1 at 2 days of age. There is marked prolongation of the QT and QTc intervals causing 2:1 block. Intravenous propranolol up to 0.3 mg/kg is administered and the QT interval increases, with no change to the 2:1 block.

Figure 2

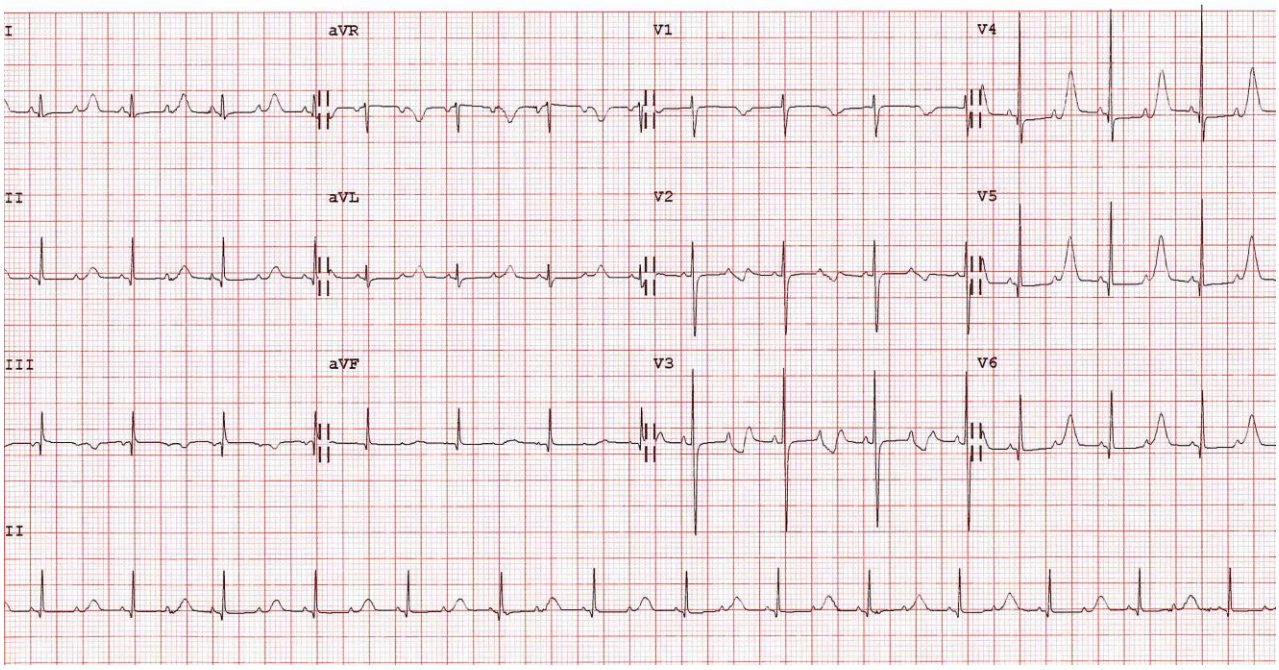

Figure 2: This ECG demonstrates a prolonged QT interval with 2:1 block causing a resultant bradycardia.

Figure 3

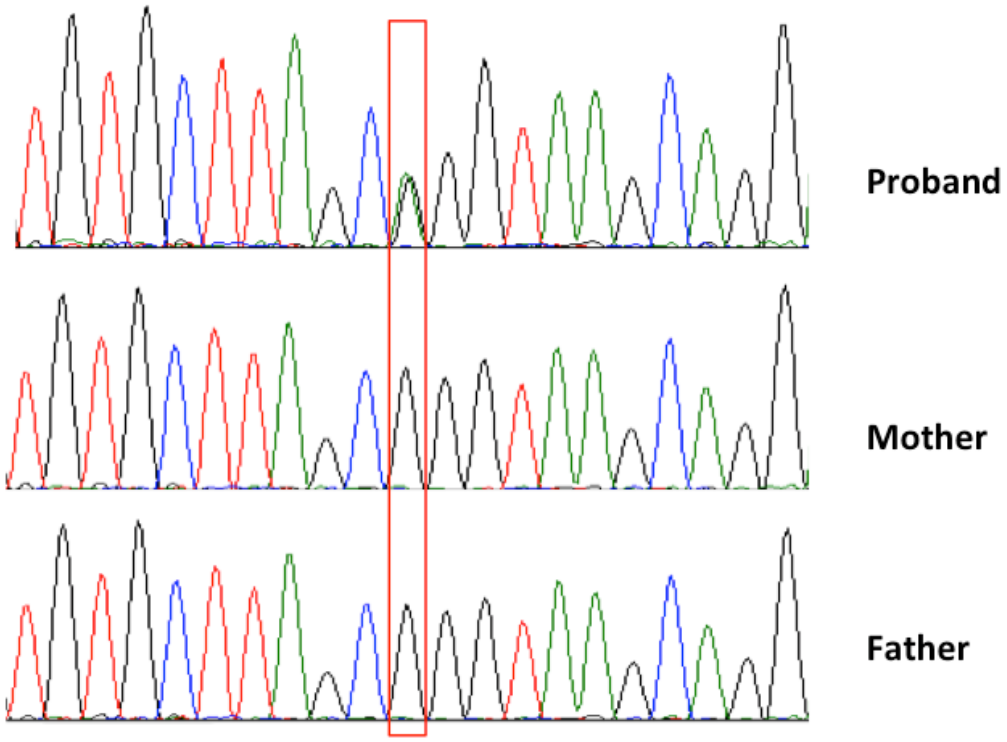

Figure 3. Sanger sequence traces showing the G to A nucleotide substitution at position c.1216 in the proband. The G to A nucleotide substitution is not detected in the traces from the mother or fathers samples, which suggests a de novo occurrence.

Figure 4

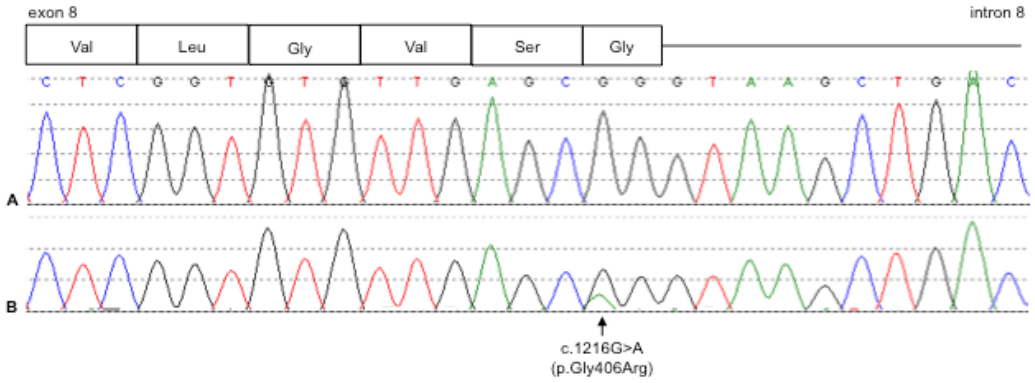

Figure 4: Electropherogram showing normal sequence of the end of exon 8 of the CACN1A gene from a control DNA sample (trace A) and DNA extracted from buccal epithelial cells from patient #5 (trace B). At base position 1216 in trace B there are two peaks, corresponding to the normal allele of the gene (G) and the mutant allele (A). In an heterozygous individual, the normal allele and mutant allele are both equally represented and the coloured peaks overlap. The marked difference in height of these peaks in trace B is consistent with the mutant allele being present at a lower level than the normal allele, indicating somatic mosaicism in buccal epithelial cells.

Figure 5

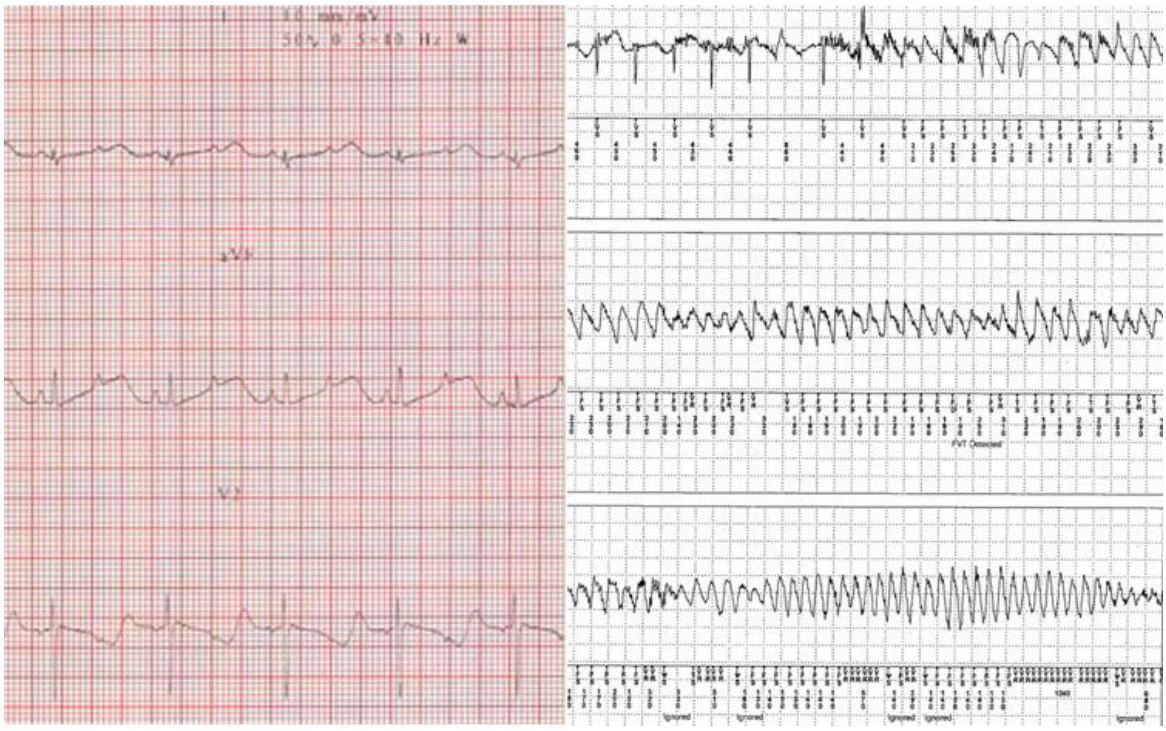

Figure 5 shows the prolonged QT interval in patient #5. On the right is the tracing from the loop recorder demonstrating torsade de pointes. An ICD was subsequently implanted.

Figure 6

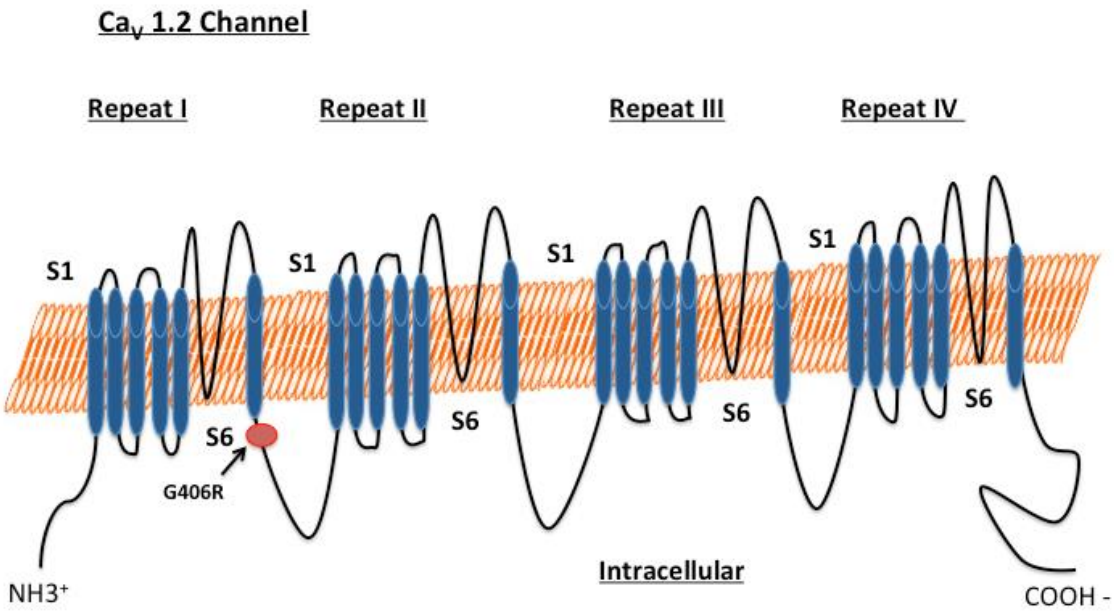

Figure 6 shows the Ca$_V$1.2 calcium channel. The most commonly seen mutation (G406R) was the known mutation in 5 out of 6 patients in our study. It is missense mutation in the transmembrane segment S6 of domain 1.

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